

CLAIMS:

5 1. A method for examining the activity of ion channels, comprising the following steps:

- providing a sample comprising ion channels; and
- determining a value of a measuring parameter as an indicator of the activity of the ion channels;

10 characterized in that said determining of the value of the measuring parameter is performed at a temperature of  $\leq$  about 10 °C.

2. The method according to claim 1, characterized in that said determining of the value of the measuring parameter is performed at a temperature of  $\leq$  about 5 °C, especially  $\leq$  about 2 °C.

15 3. The method according to claim 1 or 2, characterized in that said determining of the value of the measuring parameter is performed at a temperature of from about 10 °C to -4 °C, especially from about 5 °C to -4 °C, more preferably from about 5 °C to 0 °C, even more preferably from about 2 °C to 0 °C .

20 4. The method according to any of the preceding claims, characterized in that the sample comprises one or more cells or cell organelles which have ion channels, in particular human or animal cells or cell organelles.

5. The method according to any of the preceding claims, characterized in that the sample comprises one or more vesicles which have ion channels.

6. The method according to any of the preceding claims, characterized in that the sample comprises membrane bound ion channels, in particular ion channels embedded into a membrane of cells, cell organelles, vesicles or embedded into an artificial membrane.
- 5 7. The method according to any of the preceding claims, characterized in that said measuring parameter is a membrane potential, preferably the membrane potential of a cell, cell organelle or vesicle, or a measure of said membrane potential.
- 10 8. The method according to any of the preceding claims, characterized in that said measuring parameter is an ion concentration or a measure thereof.
- 15 9. The method according to any of the preceding claims, characterized in that the measuring parameter is an extracellular, intracellular, extravesicular and/or intravesicular ion concentration or a measure thereof.
- 10 10. The method according to any of the preceding claims, characterized in that the value of said measuring parameter is determined before, during and/or after the addition of a test substance which potentially influences the activity of the ion channels.
- 20 11. The method according to any of the preceding claims, characterized in that the activity of a transmitter-dependent ion channel is examined.
12. The method according to any of the preceding claims, characterized in that the activity of a voltage-sensitive ion channel is examined.

13. The method according to any of the preceding claims, characterized in that the activity of a potassium channel, chloride channel, sodium channel or calcium channel is examined.
- 5 14. The method according to any of the preceding claims, characterized in that said determining of a measure of the ion concentration and/or the membrane potential is effected by fluorescence methods, radioactive methods or atomic absorption spectroscopy.
- 10 15. The method according to any of the preceding claims characterized in that an optical response of (i) a carbocyanine derivative, in particular a thia-, indo-, or oxa-carbocyanine or an iodide derivative of a carbocyanine, (ii) a rhodamine dye, (iii) an oxonol dye, (iv) merocyanine 540, or (v) a styryl dye serves as a measure of the membrane potential.
- 15 16. The method according to any of the preceding claims, characterized in that the fluorescence emission of a voltage-sensitive fluorescent dye, preferably a DiBAC dye, more preferably the dye Dibac<sub>4</sub>(3), serves as a measure of the membrane potential.
17. The method according to any of the preceding claims, characterized in that the ion concentration of rubidium, especially of non-radioactive rubidium, is determined as an indicator of the activity of the ion channels.
- 20 18. The method according to any of the preceding claims, characterized in that the ion concentration, especially the ion concentration of calcium, is measured by means of chelating agents.
19. The method according to any of the preceding claims, characterized in that the values of several measuring parameters are determined.

20. The method according to any of the preceding claims for use in the research on pharmaceutically active substances, especially in the medium- or high-throughput screening of potentially or established active pharmaceutical substances, in particular the identification of potentially active pharmaceutical substances or the determination of side effects of potentially or established active pharmaceutical substances.
21. The method according to any of the preceding claims for use in the agricultural research, especially in the research on agrochemicals as e.g. insectizids.
22. Use of a voltage-sensitive or ion-sensitive indicator for the conductance of the method according to any of the preceding claims.
23. Use according to claim 22 wherein the ion-sensitive indicator is a calcium indicator, in particular a fluo-calcium indicator, a fura indicator, an indo indicator, Calcium Green™, or Oregon Green™ .
24. Use according to claim 22 wherein the ion-sensitive indicator is a sodium or potassium indicator, preferably a fluorescent sodium or potassium indicator, in particular SBF1, PBF1, Sodium Green Na<sup>+</sup> indicator, CoroNa Green Na<sup>+</sup> indicator, or CoroNa Red Na<sup>+</sup> indicator.
25. Use according to claim 22 wherein the voltage-sensitive indicator is a carbocyanine derivative, in particular an indo-, thia-, or oxa- carbocyanine or a iodide derivative of a carbocyanine; a rhodamine dye; an oxonol dye; merocyanine 540; or a styryl dye.
26. Use according to claim 25 wherein the oxonol dye is a bis-isoxazolone oxonol dye or a bis-barbituric acid oxonol (DiBAC) dye, in particular DiBAC<sub>4</sub>(3), DiSBAC<sub>2</sub>(3) or DiBAC<sub>4</sub>(5).

27. Use according to claim 25 wherein the styryl dye is an ANEP (AminoNaphthylEthenylPyridinium) dye, in particular di-4-ANEPPS, di-8-ANEPPS, di-2-ANEPEQ, di-8-ANEPPQ, di-12-ANEPPQ, di-1-ANEPIA, or a dialkylaminophenylpolyenylpyridinium dye (RH dye), in particular RH 414, RH 421, RH 795 or RH 237.
28. Use of a chelating agent for the conductance of the method according to any of claims 1 to 21.
29. Use of rubidium, in particular non-radioactive rubidium, for the conductance of the method according to any of claims 1 to 21.
30. Use of an atomic absorption spectrometer, a flow cytometer, a fluorescence microscope or fluorescence plate reader for the conductance of the method according to any of claims 1 to 21.
31. Use according to claim 30 applying a voltage-sensitive or ion-sensitive indicator according to any of claims 22 to 27, a chelating agent according to claim 28 and/or rubidium according to claim 29.